photobleaching, thus leading to a set of localizations scattered around the true position. Whereas this property does not affect the reconstruction of images of extended objects like intracellular filaments or compartments, it severely influences the interpretation and quantification of objects for which the exact stoichiometry can be important like for membrane protein aggregates. To resolve this issue, we have implemented an algorithm that uses the spatial and temporal information of fluorophore localizations from STORM/PALM experiments to obtain a quantitative picture of the underlying molecule distribution. Our algorithm reliably operates on artificial data as well as on experimental data from biological constructs with a well-defined number of attached fluorophores.

1958-Pos Board B728

Regulating Bacterial Cytokinesis: A Super-Resolution Study of ZapA and ZapB

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E. coli cell division is achieved by the concerted effort of at least 13 essential proteins that assemble at midcell in a cell-cycle dependent manner to form a macromolecular structure (divisome) capable of generating septal force. Central to divisome assembly is the initial formation of the Z-ring, a dynamic, cytokinetic suprastructure of the FtsZ protein, the prokaryotic tubulin homolog widely conserved in bacteria. Being the first division protein to localize precisely to midcell, spatial and temporal regulation of FtsZ polymerization is of critical importance to efficient proliferation.

From conventional fluorescence microscopy, the Z-ring has long been regarded as a closed-ring that circumscribes the cell. However, in vitro studies illustrated FtsZ's tendency to self-assemble into short, single-stranded protofilaments that further coalesce into multi-stranded rings and helices under molecular crowding conditions. Recently, our group has shown that the relevant in vivo structure of FtsZ is characterized by an irregular, discontinuous arrangement of overlapping protofilaments, observed in a closed ring as well as a compressed helical conformation. We believe that an equilibrium exists between the helix and ring conformations and therefore factors promoting the transition from helix to ring may serve as critical regulatory elements or pathways.

ZapA and ZapB are both non-essential, cytoplasmic proteins that associate directly with FtsZ early during division. Although their null mutants have little observable cytokinetic defect, both have a pronounced prevalence of FtsZ helical conformations, indicating their presence favors ring formation. In this study, we employ super-resolution imaging to characterize the relevant in vivo arrangements of ZapA and ZapB, as well as their relative localization with respect to FtsZ using two-color imaging methods. Lastly, by characterizing the helical conformation the Z-ring in the Zap-null mutants, we gain insight to their potential regulatory mechanism.

1959-Pos Board B729

Corral Trapping of Single Molecules in Solution: Theory and Applications

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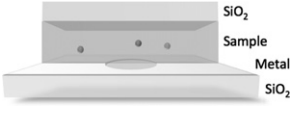
One of the great outstanding challenges for the fabrication of nanosensors and nanodevices that will drive future technologies and enable the planned assembly of molecular-scale devices is the controlled manipulation of nanoscale objects, and particularly of single molecules. Any such manipulation is preceded by an efficient, reliable method for confining (trapping) an object on demand, which has remained a formidable task in the case of single molecules.

We have successfully trapped single molecule ions and other charged particles in aqueous electrolytes using a purely electrostatic setup, demonstrating stable and reversible confinement of single and multiple particles to nanoscale dimensions over extended periods of time.

Here, we will present some of the experimental results and discuss potential mechanisms for corral trapping. Electrokinetic phenomena such as electrohydrodynamic flow, electroosmotic flow, or dielectrophoresis are known to occur near a charged metal electrode; however, we attribute corral trapping to direct charge-field interactions, i.e. and electrostatic or electrophoretic mechanism, which seems consistent with all experimental observations. Theoretical modeling of the entire setup using the finite element method will be discussed, and the limitations for applications in SNP detection and water treatment technologies will be explored.



1960-Pos Board B730

A Rejection Algorithm Essential for Quantitative Analysis in Single Molecule Super Resolution Microscopy

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Single molecule localization based super-resolution (SM-SR) imaging techniques require robust identification and accurate localization of single molecules to yield reliable emitter position estimates for further quantitative analysis. One of the key steps of SM-SR analysis is to clearly identify isolated, single emitters such that obtained localizations relate to actual single molecule locations, and are not influenced by signal from nearby or overlapping fluorophores. We refer to the process of identifying and discarding sub-regions that contain overlapping fluorophores as the rejection algorithm.

We show that even at an optimal active emitter density, more than 50% of the emitters cannot be isolated such to give an unbiased estimate of position. Furthermore, it is possible that even for the case of isolated single emitters, the fitting routine may not converge and thus provides incorrect localizations. A robust, statistically rigorous rejection algorithm that compares the fitted model to the data is necessary to avoid incorrect or biased localizations and is essential for the success of quantitative analysis.

Here, we show that the Log-likelihood Ratio (LLR) is a preferred metric for rejection algorithms and that p-values can be calculated and used for statistical significance tests when the distribution of the LLR under low signal conditions is correctly modeled. We compare the LLR with other commonly used rejection algorithms such as the elliptical or sum of squared error tests. As a demonstration, we show the improved resolution in reconstructed super-resolution images when using the LLR rejection algorithm as compared with those generated using other rejection algorithms.

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Dynamics of Nuclear Protein Exploration Revealed by Intracellular Single Particle Tracking PALM

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Cellular regulation of eukaryotic cells involves molecular interactions of factors diffusing within the cellular volume. Understanding the gene expression regulation requires thus elucidating the spatio-temporal dynamics of intranuclear proteins at the single molecule level. However, live cell imaging of single molecules in eukaryotic cells has remained mostly restricted to processes occurring in the plasma membrane, readily accessible by TIRF microscopy as opposed to intra-nuclear processes.

We report an intracellular single particle tracking method using photoactivated localization microscopy that enables the study of protein dynamics inside live eukaryotic cells. So far single particle tracking PALM (sptPALM) (Manley et al, 2008) has been restricted to cellular systems for which imaging can be performed using total internal reflection microscopy (TIRF), and believed to be limited to slow diffusing systems (~ 0.1 um2/s). Here we demonstrate an approach that reduces the background of out-of-focus fluorophores by a tight control of the photoactivation, thus allowing the detection and characterization of single protein dynamics directly in the nucleus of living cells.

Applying this method to several nuclear proteins, we captured a wide range of diffusive behaviors from very rapid diffusion (> 10 μm2/s) to bound chromatin associated states (< 0.1 μm2/s). We measured the single molecule dynamics for a diverse set of proteins, from free fluorophores (Dendra2) with no known interactions in the nucleoplasm, to DNA binding (c-Myc), RNA binding (Fibrillarin), and protein-protein interacting complexes (p-TEFb). We observe that, overall, nuclear exploration is not governed by a unique nucleoplasmic geometry but rather a protein-specific variable. Our approach provides a versatile tool for single molecule in vivo studies in eukaryotes.

References

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1962-Pos Board B732

New Tool for Single Molecule Manipulation: Optical Pushing

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The ability to measure and manipulate single molecules has greatly advanced the field of biophysics. The existing techniques, such as Optical and Magnetic Tweezers, each have their advantages and limitations. We present a proof-of-concept for an Optical Pushing apparatus, in which we exert an optical force on DNA-tethered beads with a collimated laser beam. This approach makes use of the temporal versatility of the laser power that is directly related to the force exerted on the tethered beads. This allows for fast force ramps that can be used for dynamic force spectroscopy as well as measurements on multiple single molecules at the same time. Forces achieved with the current setup agree with Mie Scattering calculations and are in range to be used for force spectroscopy on biomolecules (0.1 to 100 pN). Force calibration models used for Magnetic or Optical Tweezers are not directly applicable to our system. We therefore derive a generic, analytical model for Brownian motion of the tethered beads by solving the Langevin equation for both the translational and rotational movement. Both measured and numerically simulated data show good agreement with the analytical model. Hence, it can be used to calibrate the exerted laser force with a high accuracy, but is also applicable to other techniques, such as Tethered Particle Motion, Optical Tweezers and Magnetic Tweezers.

1963-Pos Board B733

Combining Super-Resolution Imaging and Single Particle Tracking in Living Cells to Probe Interactions Between Actin and Plasma Membrane Proteins

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Cortical actin is a complex meshwork essential for the dynamic organization and localization of plasma membrane proteins. In order to characterize dynamics of the cytoskeleton of living mammalian cells with resolution beyond the diffraction limit, we used photoactivated localization microscopy (PALM). We transfected ND7/23 and HEK 293 cells to express Dendra2 labeled actin. The cells were imaged in a custom-built total internal reflection microscope maintaining the cells under physiological conditions for prolonged periods of time. Dynamic PALM images of the actin cytoskeleton were reconstructed in a 5-second sliding time window. A resolution of 50 nm was achieved.

By combining dynamic super-resolution actin images and single particle tracking in the plasma membrane, we studied the actin cytoskeleton's role in the organization of Kv2.1 channels into segregated microdomains. Kv2.1 forms stable clusters in hippocampal neurons and transfected HEK cells, but the mechanism by which the clusters are formed is not well understood. By labeling Kv2.1 channels with quantum dots which are spectrally well separated from Dendra2, two-color images were obtained which revealed complex interactions between the actin cytoskeleton and Kv2.1 channels. This work demonstrates that PALM combined with single particle tracking is an effective technique to probe the dynamic interactions between cortical actin and membrane proteins.

1964-Pos Board B734

Two- and Three-Dimensional Single-Molecule Super-Resolution Imaging in Live Bacteria Cells

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Single-molecule imaging has extended the resolution of fluorescence microscopy down to the nanometer scale. This super-resolution technique is non-invasive, tolerates simple sample preparation, and takes advantage of high-specificity labeling schemes. We will discuss recent studies focused on imaging protein structure and dynamics in live bacterial cells, with attention to the particular challenges that this system presents: the organisms are small, have short cell cycles, live in specific environments, and their organization is relatively poorly understood. We have developed methods to extend the capabilities of single-molecule fluorescence microscopy to study motion and localization in live *Caulobacter crescentus*, *Vibrio cholerae*, and *Bacteroides thetaiotaomicron*.

FtsZ is an essential protein that polymerizes at the mid-cell, recruits the division machinery, and may generate constrictive forces necessary for cytokinesis. Based on astigmatism and on the natural dynamics of the protein, we resolve in two and three dimensions the midplane Z-ring formed by FtsZ, in *C. crescentus*. We further apply live-cell single-molecule imaging to two prokaryotes of biomedical interest, *V. cholerae*, and the gut symbiont *B.thetaiotaomicron*. The *V. cholerae* protein TcpP is a rare example of a membrane-bound transcription factor, and we have investigated the dynamics and localization of TcpP, its binding partner ToxR, and the *toxT* gene they regulate, in order to elucidate the mechanism of membrane-bound transcription activation. The starch utilization system (Sus) allows *B. thetaiotaomicron* to catabolize complex carbohydrates, and we have investigated the response of Sus proteins to stimuli with live anaerobic cell single-molecule imaging.

1965-Pos Board B735

Introducing the Electromagnetic Torque Tweezers

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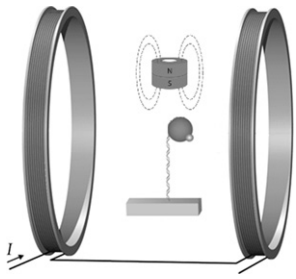
Most single-molecule manipulation methods, including atomic force microscopy, optical tweezers, and magnetic tweezers, report on parameters such as force and extension. Recently several techniques have been developed that permit one to additionally monitor rotational motion and torque. Magnetic torque tweezers (MTT) (Lipfert et al., Nature Methods 2010) are one such technique. Current MTT schemes however have the drawback that the force and torque degrees of freedom are intrinsically coupled.

Here, we present the electromagnetic torque tweezers (eMTT), a novel instrument that decouples the force and torque degrees of freedom. The eMTT combines a vertically-oriented, cylindrically-shaped permanent magnet that provides a strong field gradient and enables the application of a stretching force, with two pairs of Helmholtz geometry that produce nearly homogenous magnetic fields in the horizontal plane. We use these in-plane fields to rotate the bead about the vertical axis, to accurately set the stiffness of the magnetic torsional trap, and to vary its stiffness at will. We fully calibrate and characterize the eMTT, showing how it allows one to span the range between conventional and freely orbiting magnetic tweezers with the turn of a knob, and demonstrate its implementation on DNA and other biopolymers.



1966-Pos Board B736

Three Dimensional Tethered Particle Motion for DNA-Protein Interactions Studies

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We developed a unique optical system for studying the interaction of proteins with DNA based on tethered particle motion (TPM). The method uses gold nano-beads and total internal reflection (TIR) illumination and allows recording the DNA dynamics in three dimensions (3D).

We studied the 3D end-to-end distribution of a tethered DNA. We measured the axial distribution that was never measured before [1] and confirmed the theoretical solution but emphasize the invalidity of the Gaussian random walk approximation for short DNA strands.

We also measured the interaction of HU protein with DNA and confirmed its bi-modal effect on DNA as a function of the HU concentration [2]. To the best of our knowledge, this is the first time that TPM is used for actual DNA-protein interaction studies (except for looping).

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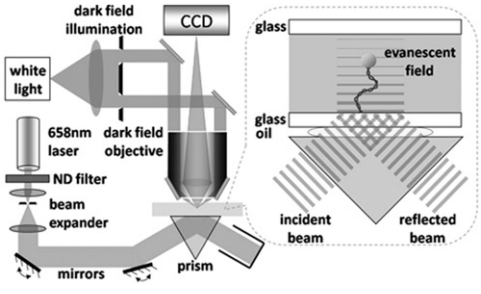

1967-Pos Board B737

Imaging Protein in Water with Nanometer Resolution

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